Claims

We claim:

1. A method for evaluating a biological sample for the presence or absense of human parainfluenza virus 1 infection, comprising the steps of

(a) isolating RNA from a biological sample,

(b) creating cDNA from the isolated RNA,

(c) exposing the cDNA to a pair of oligonucleotide primers selected from SEQ ID NOs:1-9 or inverse complements of SEQ ID NOs:1-9 under conditions in which the primers will amplify a human parainfluenza virus 1 sequence if the human parainfluenza virus 1 is present, wherein an amplification product is produced if the human parainfluenza virus 1 is present; and

(d) determining whether the amplified product is present.

2. A method for evaluating a biological sample for the presence or absence of human parainfluenza virus 1 infection comprising the steps of

(a) isolating RNA from a biological sample,

(b) exposing the RNA to a labelled probe selected from SEQ ID NOs:1-9 under conditions wherein a hybridization reaction between the probe and a complementary sequence can occur, and

(c) examining the reaction to determine whether a hybridization product exists.

3. The method of claim 1 wherein the biological sample is a respiratory secretion.

4. The method of claim 1 wherein step (d) comprises attaching the product of step (c) to a marker enzyme and examining the products of step (c) for the marker.

5. The method of claim 6 wherein the marker enzyme is horse radish peroxidase.

6. The method of claim 2 wherein the primers or probes are SEQ ID NOs:19 and 20.

7. The method of claim 1 additionally comprising the step of attaching the amplification product to a solid support.

8. The method of claim 8 wherein the solid support is a microtiter dish.

9. The method of claim 1 wherein the amplification product is detected by hybridization of an oligonucleotide probe specific for a hemagglutinin neuraminidase sequence.

10. The method of claim 9 wherein the oligonucleotide probe is labelled with an enzyme.

11. A kit for the detection of human parainfluenza virus 1 comprising a pair of primers selected from SEQ ID NOs:1-9.

12. The kit of claim 11 further comprising a pair of primers specific for human parainfluenza virus 2 and a pair of primers specific for human parainfluenza virus 3.

13. The kit of claim 11 additionally comprising a HN-specific oligonucleotide probe.

14. A method of detecting human parainfluenza virus infection in a biological sample comprising the steps of

(a) isolating RNA from a biological sample,

(b) creating cDNA from the isolated RNA,

(c) exposing the cDNA to a primer pair specific for a human parainfluenza sequence under conditions suitable for nucleic acid amplification, wherein an amplification product is formed if the sample contains human parainfluenza virus, and

(d) determining whether the amplification product is present by exposing the step (c) products to protein-linked oligonucleotide probes under conditions suitable for hybridization between complementary nucleic acid sequences and

examining the probes for the presence of a hybridization product, wherein the oligonucleotide probe is of a sequence identical to a human parainfluenza sequence and wherein the oligonucleotide probe is attached to a solid support.

15. The method of claim 14 wherein the oligonucleotide probe is linked to BSA.

16. The method of claim 15 wherein at least one primer of the primer pair is enzymatically labelled.

17. A kit for the method of claim 14 comprising a pair of primers specific for a human parainfluenza sequence and a protein-linked oligonucleotide probe specific for a human parainfluenza sequence.

18. The kit of claim 17 wherein at least one of the primers is labelled.

19. A method for evaluating a biological sample for multiple virus infection comprising the steps of

(a) isolating RNA from a biological sample,

(b) exposing the RNA to at least one labelled probe selected from SEQ ID NOs:1-9 and to labelled probes designed to hybridize to respiratory syncytial virus or influenza virus under conditions wherein a hybridization reaction between the probe and a complementary sequence can occur, and

(c) examining the reaction to determine whether a hybridization product exists.

20. A method for evaluating a biological sample for the presence or absence of multiple virus infections, comprising the steps of

(a) isolating RNA from a biological sample,

(b) creating cDNA from the isolated RNA,

(c) exposing the cDNA to a pair of oligonucleotide primers selected from SEQ ID NOs:1-9 or inverse complements of SEQ ID NOs:1-9 under conditions in which the primers will

amplify a human parainfluenza virus 1 sequence if the human parainfluenza virus 1 is present, wherein an amplification product is produced if the human parainfluenza virus 1 is present;

(d) exposing the cDNA to at least one additional pair of oligonucleotide primers under conditions designed to amplify respiratory syncytial virus or influenza virus sequences if the viruses are present, wherein an amplification product is produced if the respiratory syncytial virus or influenza virus are present; and

(e) determining whether the amplified products are present.

21. The method of claim 20 additionally comprising the step of exposing the cDNA to primer pairs specific for human parainfluenza virus 2 and human parainfluenza virus 3.

22. The method of claim 20 wherein the biological sample is examined for both respiratory syncytial virus and influenza virus.

23. The method of claim 22 wherein the biological sample is examined for respiratory syncytial viruses A and B and influenza viruses A and B.

24. The method of claim 23 wherein the primers are selected from the group consisting of SEQ ID NOs:30, 31, 33, 34, 36, 37, 39, 40, 42, 43, 45, 46, 48, 49, 51, 52, 54, 55, 57 and 58.

25. The method of claim 20 wherein the biological sample is a respiratory secretion.

26. The method of claim 20 wherein step (e) comprises attaching the products of steps (c) and (d) to a marker enzyme and examining the products of steps (c) and (d) for the marker.

27. The method of claim 23 wherein step (e) comprises exposing the results of steps (c) and (d) to oligonucleotide probes specific for human parainfluenza virus 1, 2 and 3, respiratory syncytial virus A and B and influenza virus A and B.

28. The method of claim 20 wherein the biological sample is evaluated for the presence or absence of human parainfluenza virus-2 and 3.

29. The method of claim 28 wherein the biological sample is evaluated for the presence of respiratory syncytial viruses A and B and influenza viruses A and B.

30. The method of claim 29 wherein the probes are selected from the group consisting of SEQ ID NOs:32, 35, 38, 41, 44, 47, 50, 53, 56 and 59.

31. The method of claim 20 additionally comprising the step of attaching the amplification product to a solid support.

32. The method of claim 31 wherein the solid support is a microtiter dish.

33. The method of claim 20 wherein the amplification product is detected by hybridization of an oligonucleotide probe specific for a hemagglutinin neuraminidase sequence.

34. The method of claim 33 wherein the oligonucleotide probe is labelled with an enzyme.

35. A kit for the detection of multiple viruses comprising a pair of primers selected from SEQ ID NOs:1-9 and at least one pair of primers designed to amplify respiratory syncytial virus A or B or influenza virus A or B.

36. The kit of claim 35 additionally comprising a HN-specific oligonucleotide probe.

37. The kit of claim 35 further comprising a pair of primers specific for human parainfluenza virus 2 and a pair of primers specific for human parainfluenza virus 3.

38. The kit of claim 37 further comprising primers specific for respiratory syncytial virus A and B and influenza virus A and B.

39. The kit of claim 38 wherein the primers are selected from SEQ ID NOs:30, 31, 33, 34, 36, 37, 39, 40, 42, 43, 45, 46, 48, 49, 51, 52, 54, 55, 57 and 58.

40. The kit of claim 38 additionally comprising oligonucleotide probes specific for the HN gene of HPIV1, 2 or 3, the influenza A virus M gene, the influenza virus B NS gene, the respiratory syncytial virus 1B, N and F genes, and the RSVB 1B, N and G genes.

41. The kit of claim 40 wherein the probes are selected from SEQ ID NOs:32, 35, 38, 41, 44, 47, 50 53, 56 and 59.

42. The kit of claim 39 additionally comprising a pair of primers specific for human parainfluenza virus 2 and a pair of primers specific for human parainfluenza virus 3.

43. The kit of claim 39 wherein the HN-specific oligonucleotide probe is labelled.

44. The kit of claim 39 wherein the HN-specific oligonucleotide probe is linked to a protein suitable for attaching the probe to a solid support.

45. The kit of claim 39 wherein the HN-specific oligonucleotide probe is labelled.

46. A method of detecting multiple virus infection in a biological sample comprising the steps of

(a) isolating nucleic acid from a biological sample,

(b) exposing the nucleic acid or cDNA created from the nucleic acid to primer pairs specific for human parainfluenza virus-1, 2 and 3, respiratory syncytial virus A and B and influenza virus A and B sequences under conditions suitable for nucleic acid amplification, wherein an amplification product is formed if the sample contains any of the viruses, and

(c) determining whether the amplification product is present by exposing the step (b) products to protein-linked oligonucleotide probes under conditions suitable for hybridization between complementary nucleic acid sequences and examining the probes for the presence of a hybridization product, wherein the oligonucleotide probe is of a sequence identical to a viral sequence.

47. A kit for the method of claim 46 comprising a pair of primers and probes specific for human parainfluenza virus-1, 2 and 3, respiratory syncytial virus A and B and influenza virus A and B.

48. A method for PCR amplification of a nucleic acid molecule, comprising the improvement of supplying 5' and 3' primers of unequal concentrations.

49. The method of claim 48 wherein the ratio of 5' to 3' primer is selected from the group consisting of approximately 50:25, 25:50, 12.5:50 and 12.5:25.

50. A method of PCR amplification of a nucleic acid molecule comprising the improvement of denaturing the reaction mix, comprising a 5' and a 3' primer and a template nucleic acid molecule, at least two times.

51. The method of claim 50 wherein the reaction mix is denatured at least four times for 5 minutes at 95°C.